JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1445–1447 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1445–1447.2005

Comparison of COBAS AMPLICOR *Neisseria gonorrhoeae* PCR, Including Confirmation with *N. gonorrhoeae*-Specific 16S rRNA PCR, with Traditional Culture

Dirk S. Luijt,[1] Petra A. J. Bos,[1] Anton A. van Zwet,[1] Pieter C. van Voorst Vader,[2] and Jurjen Schirm[1]*

Regional Public Health Laboratory[1] and Department of Dermatology, University Hospital Groningen,[2] Groningen, The Netherlands

Received 28 May 2004/Returned for modification 23 August 2004/Accepted 22 October 2004

A total of 3,023 clinical specimens were tested for *Neisseria gonorrhoeae* by using COBAS AMPLICOR (CA) PCR and confirmation of positives by *N. gonorrhoeae*-specific 16S rRNA PCR. The sensitivity of CA plus 16S rRNA PCR was 98.8%, compared to 68.2% for culture. Confirmation of CA positives increased the positive predictive value from 54.8 to 96.6%.

For the detection of *Neisseria gonorrhoeae*, culture is still used by many laboratories. However, recent studies showed that nucleic acid amplification-based *N. gonorrhoeae* tests (NAATs) are much more sensitive (1, 2, 4, 6, 7, 10). The Roche COBAS AMPLICOR (CA) *N. gonorrhoeae* test is probably the most widely used amplification test for *N. gonorrhoeae*. It was shown that certain strains of *N. subflava*, *N. cinerea, N. flavescens, N. lactamica, N. sicca,* and *Lactobacillus* species may produce false-positive results with the CA *N. gonorrhoeae* test (3, 4, 5, 9, and Manual for COBAS AMPLICOR Neisseria gonorrhoeae PCR test, version 1.0, Roche Diagnostics) and some other *N. gonorrhoeae* NAATs (8). Therefore, it is clear that positive results obtained with the CA *N. gonorrhoeae* test should be confirmed by another test method (2, 3, 4, 8, 9). In the present study we evaluated the reliability of *N. gonorrhoeae* testing in a low-prevalence population, using the CA *N. gonorrhoeae* test as a screening assay and an *N. gonorrhoeae*-specific 16S rRNA PCR for confirmation.

N. gonorrhoeae PCR and culture were performed on 3,023 specimens from nonselected patients (2,415 female and 608 male) obtained by visiting general practitioners (48%), general hospitals (32%), or venereal disease (VD) clinics (20%). The vast majority of the patients were symptomatic, and 75% of them were between 15 and 35 years of age. Over 95% of the specimens were urogenital swabs, and the remaining 5% were rectal, throat, or eye swabs. Swabs for PCR and culture were sent to the laboratory in 2-sucrose-phosphate medium and in charcoal transport medium, respectively. The CA *N. gonorrhoeae* PCR (Roche) was tested according to the manufacturer's instructions (Roche Diagnostics manual, version 1). A specimen was considered negative for *N. gonorrhoeae* if the optical density (OD) was <0.200 and the OD of the inhibition control (IC) was ≥ 0.200. The specimen was considered positive for *N. gonorrhoeae* if the OD was ≥ 0.200, regardless of the IC result. All specimens with an initial positive result were retested in the CA *N. gonorrhoeae* PCR, and the result of this repeat CA test was conclusive for the final interpretation of the CA *N. gonorrhoeae* test. For the 16S rRNA *N. gonorrhoeae* PCR test, new DNA extracts from the original clinical specimens were prepared. The 16S rRNA *N. gonorrhoeae* PCR test (Roche) was also performed according to the manufacturer's instructions (manual for Neisseria gonorrhoeae 16S rRNA PCR test, version 2, Roche Diagnostics) and included hybridization with an *N. gonorrhoeae*-specific probe added to microwell plates. Specimens repeatedly positive by CA *N. gonorrhoeae* PCR and positive by 16S rRNA *N. gonorrhoeae* PCR were considered to be *N. gonorrhoeae* PCR positive. All other specimens were *N. gonorrhoeae* PCR negative. For culture, the specimens were inoculated on chocolate agar and chocolate agar with vancomycin–trimethoprim–colistin-sulfate–amphotericin. The media were incubated at 37°C with 5 to 10% CO_2 for 2 days and then were examined for *N. gonorrhoeae*-like colonies. Suspected colonies were further examined by an oxidase test and gram staining and were also subcultured. The results of all PCR tests were compared with the results of *N. gonorrhoeae* culture. Discordant specimens, which were *N. gonorrhoeae* PCR positive and culture negative, were further investigated using the ligase chain reaction (LCR) *N. gonorrhoeae* test (Abbott Laboratories). True *N. gonorrhoeae*-positive specimens were all specimens which were positive by *N. gonorrhoeae* culture as well as those additional specimens which were repeatedly positive by the CA *N. gonorrhoeae* PCR, and by 16S rRNA *N. gonorrhoeae* PCR, and by the LCR *N. gonorrhoeae* assay. All other specimens were defined as true *N. gonorrhoeae* negative.

A total of 190 out of 3,023 (6.3%) specimens were initially positive by CA. After retesting, only 155 out of 190 (81.6%) of these remained positive. The reason for this discrepancy is unknown. A similar lack of reproducibility was described earlier for CA *Chlamydia trachomatis* PCR (11). Of the 155 repeatedly CA *N. gonorrhoeae* PCR-positive specimens, 87 (56.1%) were also positive by the 16S rRNA *N. gonorrhoeae* PCR. Only 57 (65.5%) of these 87 *N. gonorrhoeae* PCR positives were culture positive. In addition, one of the *N. gonorrhoeae* PCR-negative samples gave a positive culture. Further

* Corresponding author. Mailing address: Regional Public Health Laboratory, van Ketwich Verschuurlaan 92, 9721 SW Groningen, The Netherlands. Phone: 31-50-5215160. Fax: 31-50-5271488. E-mail: j.schirm@slgd.nl.

TABLE 1. Test characteristics

Specimen type[c] and/or criterion	Value for test method		
	N. gonorrhoeae culture	CA *N. gonorrhoeae* PCR[a]	Final *N. gonorrhoeae* PCR result[b]
True positive			
No. of positive results	58	85	84
No. of negative results	27	0	1
True negative			
No. of positive results	0	70	3
No. of negative results	2,938	2,868	2,935
Sensitivity (%)	68.2	100	98.8
Specificity (%)	100	97.6	99.9
Positive predictive value (%)	100	54.8	96.6
Negative predictive value (%)	99.1	100	100

[a] Only specimens which were repeatedly reactive in the CA *N. gonorrhoeae* PCR test were considered to be CA *N. gonorrhoeae* PCR positive.

[b] Specimens which were repeatedly positive by CA *N. gonorrhoeae* PCR and positive by 16SrRNA *N. gonorrhoeae* PCR were considered to be *N. gonorrhoeae* PCR positive. All other specimens were *N. gonorrhoeae* PCR negative.

[c] Specimens were considered to be true *N. gonorrhoeae* positive or true *N. gonorrhoeae* negative as described in the text.

analyses of the 30 samples that were *N. gonorrhoeae* PCR positive and *N. gonorrhoeae* culture negative by LCR resulted in 27 additional true-positive specimens.

The overall prevalence of *N. gonorrhoeae* in this study was 2.8%, but with urogenital specimens there were large differences between women (1.2%) and men (8.9%) ($P < 0.001$, Pearson's chi-squared test). Table 1 shows the overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each method. Without confirmation by 16S rRNA *N. gonorrhoeae* PCR, the PPV of CA *N. gonorrhoeae* PCR on all samples was only 54.8%. For urogenital specimens from women and men, the PPV of the CA *N. gonorrhoeae* PCR was 43.3 and 96.0%, respectively. Recently, Diemert et al. (3) showed that additional confirmatory PCR was necessary by comparing *N. gonorrhoeae* culture and CA *N. gonorrhoeae* PCR in 737 samples in a very-low-prevalence setting of 0.5%. However, they did not further investigate the validity of their 16S rRNA *N. gonorrhoeae* PCR confirmation assay for the discordant specimens that were *N. gonorrhoeae* PCR positive but culture negative. Martin et al. (6) and Van Der Pol et al. (9) described another algorithm to increase the PPV of the CA *N. gonorrhoeae* test: retesting in duplicate, depending on the initial OD value. When we applied this algorithm to our data, the PPV of the CA *N. gonorrhoeae* PCR was 65.9%, which is much lower than the PPV of at least >90% described by Van Der Pol et al. (9).

Of the 70 CA *N. gonorrhoeae* PCR false-positive specimens, 30 (42.9%) were not obtained from the urogenital tract but were mainly from throat specimens ($n = 21$). The inclusion of these nonurogenital specimens influenced the PPV of the CA *N. gonorrhoeae* PCR, being 65.8% for urogenital specimens and 21.1% for nonurogenital specimens. The most likely explanation for the high percentage of false positives with throat swabs is cross-reaction with *N. cinerea* and *N. subflava* (3, 4, 5, 9, and Roche Diagnostics manual, version 1). Most of the remaining false-positive reactions may be explained by cross-reactions with *Lactobacillus* species, which belong to the normal flora of the female genital tract (4).

The present study shows that CA *N. gonorrhoeae* PCR plus 16S rRNA *N. gonorrhoeae* PCR confirmation is much more sensitive (98.8%) than culture (68.2%) ($P < 0.001$, Fisher's exact test). Moreover, the enormous surplus of positive results was very specific, as was shown by the additional confirmation by LCR. The low sensitivity of culture compared to that of PCR could not be explained by suboptimal transport conditions, because culture results obtained with specimens collected very close to the laboratory did not significantly differ from the results obtained with specimens collected at much larger distances (data not shown).

Because the sensitivity of culture might be dependent on the presence of clinical symptoms, we investigated the clinical data of all 18 *N. gonorrhoeae*-positive patients from one of our hospitals. It appeared that 7 of 8 (87.5%) patients with clinical symptoms and only 2 of 10 (20%) patients without symptoms were *N. gonorrhoeae* culture positive ($P = 0.004$, Pearson's chi-squared test).

From 18 *N. gonorrhoeae* PCR-positive patients we received a follow-up specimen 13 to 35 days (average, 24 days) after antibiotic treatment. None of these follow-up specimens were positive for *N. gonorrhoeae* by PCR. Therefore, *N. gonorrhoeae* PCR can also be used for follow-up treatment. Only when susceptibility testing is required does *N. gonorrhoeae* culture remain necessary.

In conclusion, due to its high sensitivity, *N. gonorrhoeae* PCR (or perhaps another NAAT) should replace *N. gonorrhoeae* culture, especially when asymptomatic patients are tested. CA *N. gonorrhoeae* PCR is a very useful screening assay due to its semiautomatic platform, making it much easier to perform bulk testing, and to its ability to test for *Chlamydia trachomatis* simultaneously. However, CA *N. gonorrhoeae* PCR-positive results should always be confirmed with an alternative PCR test, such as the 16S rRNA PCR, especially when low-prevalence populations or nonurogenital specimens are tested.

We thank S. Bruisten (GG and GD, Amsterdam, The Netherlands) for performing the LCR assay on discrepant specimens. We also thank J. Moedt and I. K. Roozeboom-Roelfsema for excellent technical assistance.

REFERENCES

1. **Carroll, K. C., W. E. Aldeen, M. Morrison, R. Anderson, D. Lee, and S. Mottice.** 1998. Evaluation of the Abbott Lcx ligase chain reaction assay for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine and genital swab specimens from a sexually transmitted disease clinic population. J. Clin. Microbiol. **36:**1630–1633.
2. **Crotchfeld, K. A., L. E. Welsh, D. DeBonville, M. Rosenstraus, and T. C. Quinn.** 1997. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. J. Clin. Microbiol. **35:**1536–1540.
3. **Diemert, D. J., M. D. Libman, and P. Lebel.** 2002. Confirmation by 16S rRNA PCR of the COBAS AMPLICOR CT/NG test for diagnosis of *Neisseria gonorrhoeae* infection in a low-prevalence population. J. Clin. Microbiol. **40:**4056–4059.
4. **Doornum, G. J. J., L. M. Schouls, A. Pijl, I. Cairo, M. Buimer, and S. Bruisten.** 2001. Comparison between the LCx probe system and the COBAS AMPLICOR system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands. J. Clin. Microbiol. **39:**829–835.
5. **Farrell, D. J.** 1999. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using cppB nested PCR and 16S rRNA PCR. J. Clin. Microbiol. **37:**386–390.
6. **Martin, D. H., C. Cammarata, B. Van Der Pol, R. B. Jones, T. C. Quinn,**

C. A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D. Jungkind, B. Turner, and C. Peyton. 2000. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae*. J. Clin. Microbiol. **38:**3544–3549.

7. **Moncada, J., J. Schachter, E. W. Hook III, D. Ferrero, C. Gaydos, T. C. Quinn, D. Willis, A. Weissfeld, and D. H. Martin.** 2004. The effect of urine testing in evaluations of the sensitivity of the Gen-Probe APTIMA Combo 2 Assay on endocervical swabs for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Sex. Trans. Dis. **31:**273–277.
8. **Palmer, H. M., H. Mallinson, R. L. Wood, and A. J. Herring.** 2003. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **41:**835–837.
9. **Van Der Pol, B., D. H. Martin, J. Schachter, T. C. Quinn, C. A. Gaydos, R. B. Jones, K. Crotchfelt, J. Moncada, D. Jungkind, B. Turner, C. Peyton, J. F. Kelly, J. B. Weiss, and M. Rosenstraus.** 2001. Enhancing the specificity of the COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae* by retesting specimens with equivocal results. J. Clin. Microbiol. **39:**3092–3098.
10. **Van Der Pol, B., D. V. Ferrero, L. Buck-Barrington, E. Hook III, C. Lenderman, T. C. Quinn, C. A. Gaydos, J. Lovchik, J. Schachter, J. Moncada, G. Hall, M. J. Tuohy, and R. B. Jones.** 2001. Multicenter evaluation of the BDProbeTec ET system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. J. Clin. Microbiol. **39:**1008–1016.
11. **Vincelette, J., J. Schirm, M. Bogard, A.-M. Bourgault, D. S. Luijt, A. Bianchi, P. C. van Voorst Vader, A. Butcher, and M. Rosenstraus.** 1999. Multicenter evaluation of the fully automated COBAS AMPLICOR PCR test for detection of *Chlamydia trachomatis* in urogenital specimens. J. Clin. Microbiol. **37:**74–80.